REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks are respectfully requested.

Claims 7-21 and 23-32 are currently pending.

Claims 7, 20 and 30-32 have been amended herein. New claims 33-35 have been added, reciting the subject matter of claims 30-32 as dependent of claim 20. Basis for the amendments and the new claims may be found throughout the application and specification as-filed, especially on page 5, lines 20-34 (activation of blood vessels) and pages 3-4 (discussing the types of tissues which may be treated by the present invention, including those which are non-malignant).

Claim 29 has been canceled by way of the present Amendment. Applicants reserve the right to file a continuation or divisional application directed to any subject matter deleted by way of this Amendment.

Rejections under 35 U.S.C. § 112

Claims 7-12 stand rejected under 35 U.S.C. § 112, first paragraph, as purportedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Specifically, the Office Action states that the specification fails to teach which of the fragments from a 43-46 kD protein would still have a proper function of the full length TF as well as the core structures related to such a function. The Office Action further states that the protein

chemistry art is unpredictable, and that an adequate written description for a functional protein requires more than a mere statement that it is part of the invention.

The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter, rather than the presence or absence of literal support in the specification for the claim language. *In re Kaslow*, 217 USPQ 1059, 1076 (Fed. Cir. 1983). *Ex parte Remark*, 15 USPQ2d 1498, 1506 (PBAI 1990). Further, Applicants submit that the knowledge and level of skill in the art is such that one skilled in the art would be able envisage the claimed invention from the present disclosure.

Functional fragments of tissue factor are well known

Applicants submit that there is a large amount of well known literature and data available on the topic of functional fragments of tissue factor (TF). This data includes substantial evidence showing that the full protein is not required for complete TF function. In fact, characterized fragments are completely sufficient. As early as 1987, four independent groups cloned the 2.3 kb TF cDNA. Since then, a large number of scientists have explored this field and have shown that a unique 12.4 kb TF gene on human chromosome 1 is organized into six exons, whereby the second through fifth exons encode the extracellular domain of the protein and the sixth exon provides both the transmembrane and the cytoplasmic domains. Furthermore, it has been well documented that the TF protein is a 295 residue glycoprotein which is processed to remove the 32 leader peptide.

In its mature form, TF consists of a 219 amino acid extracellular domain, a 23 amino acid transmembrane segment and a 21 amino acid cytoplasmic tail. The primary structure of TF has been derived and confirmed by comparison to protein and peptide sequence data. Not only are these facts well known, but even the TF crystal structure has been deduced. Analysis of the crystal structure revealed that it is composed of two fibronectin type-III domains connected to each other at an angle of about 120 degrees. A large number of studies have explored the crystal structure of TF, particularly the extracellular region of TF and the complex of TF and VIIa.

Mapping of the amino acids involved in binding of FVII to TF and activation of this macromolecular substrate immediately followed the cloning of TF. The first approach was by mapping of competitive antibody binding epitopes and chemical crosslinking. Later studies involved proteolytic fragmentation of TF and alanine mutant scanning of large regions of the TF extracellular domain.

A broad foundation of knowledge regarding the structure and functionality of TF has been well known, allowing for researchers to understand and predict the function of TF domains and fragments. Thus, it is no surprise that these predictions based on fragments could be subsequently confirmed by a large number of scientific groups in addition new information was obtained by further exploration of the effect. Post-translational modifications of the TF molecules have been well characterised structurally, and functional studies investigated the effects on ligand binding and activity. In order to investigate this topic full length tissue factor and three truncated forms were expressed in human kidney cells. Three TF variants were constructed:

The first variant lacks the cytoplasmic tail, the second variant lacks both the transmembrane domain and the cytoplasmic tail, and the third variant, known as TF-P1, contains the extracellular domain of TF (the first 219 amino acids) fused to the last 37 amino acids of decayaccelerating factor (DAF), a phosphatidylinositol (P1)-anchored membrane protein. The activity of these variants was assessed by measuring factor X activation. The first variant, which lacks the cytoplasmic tail, is fully functional and has a specific activity comparable with that of the full length molecule, whereas the second variant which lacks both the transmembrane and cytoplasmic domain, is not functional. Furthermore, the P1 anchor in the third variant, restores TF activity lost when the transmembrane domain is deleted from variant 2. These results demonstrate that while the transmembrane domain of TF is not required, there is an absolute requirement of lipid association for TF activity (Paborsky *et al.*, *The Journal of Biological Chemistry,* 266 (32): 21911-21916, 1991). These studies suggested that the N-terminal part of the TF extracellular domain is essential for the expression of activity of the TF-FVIIa complex. Moreover, the experiments demonstrated that the C-terminal part of the TF extracellular domain plays a role in FVII binding. A study on the catalytic function of a truncated TF-fragment was carried out by Ruf *et al.* (*Journal of Biol Chem,* 266 (4): 2158-2166, 1991). A recombinant TF mutant, deleted of membrane spanning and intracellular domains, was used to evaluate the role of phospholipid interactions for assembly of substrate with the catalytic TF-VIIa complex. The studies provide evidence for catalytic function of TFVIIa independent of assembly on phospholipid and further demonstrate that the primary protein:

protein interactions of VIIa with the surface domains of TF alone are sufficient for marked enhancement of the catalytic function of VIIa.

Thus, it is clear that sufficient information is available for the skilled artisan to envision the detailed structure of the polypeptides recited in the claims and to understand if they can serve as a functional TF. Moreover, a wide variety of information can be found in the literature, demonstrating which modifications and variations can be tolerated in this protein and still allow proper TF function. In fact, the cited reference McDonald *et al.* (US 6,120,799), as cited in the present Office Action, states that the genetic material encoding truncated TF and other factors is known.

In support of the above arguments that functional tissue factor is well known, Applicants submit that following references for consideration. All of the references pre-date the filing of the present application.

- Banner *et al.* discuss the crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. *Nature*, 380 (6569): 21-23, 1996.
- Bazan discusses the structural design and molecular evolution of the cytokine receptor superfamily. *Proc. Natl. Acad. Sci,* USA 87: 6934 - 6938, 1990.
- Camerer *et al.* discuss the cell biology of tissue factor as the principal initiator of blood coagulation. *Thromb Res,* 81(1): 1-41, 1996.
- Edgington *et al.* discuss the structural biology of expression and function of tissue factors. *Thromb Res* 66: 67-69, 1991.

- Gibbs *et al.* discuss the identification of the Factor VIIa binding site on TF by homologous loop swap and alanine scanning mutagenesis. *Biochemistry,* 33: 14003-14010, 1994.
- Harlos *et al.* discuss the crystal structure of the extracellular region of human tissue factor. *Nature* 370 (6491):662-6. 1994.
- Hasselbacher *et al.* disclose the environments of the four typtophans in the extracellular domain of human tissue factor, and provide a comparison of results from absorption and fluorescence difference spectra of tryptophan replacement mutants with crystal structure of the wild-type protein, *Biophys J,* 69 (1): 20-29, 1995.
- Huang *et al.* disclose experiments on tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. *Science,* 275 (5299): 547-550, 1997.
- Mackman discusses the regulation of the TF gene. *FASEB J*, 9: 883-639, 1995.
- Mackman *et al.* disclose the complete sequence of the human TF gene, as a highly regulated cellular receptor that initiates the coagulation protease cascade. *Biochemistry*, 28: 1755-1762, 1989.
- Muller *et al.* disclose the crystal structure of the extracellular domain of human tissue factor refined to 1.7 A resolution. *J Mol Biol*, 256 (1): 144-159, 1996.

- O'Brien *et al.* disclose the structural requirements for the interaction between tissue factor and factor VII. *Biochem J* 292: 7-12, 1993.
- Paborsky *et al.* disclose that lipid association, but not the transmembrane domain, is required for TF activity. *The Journal of Biological Chemistry,* 266 (32): 21911-21916, 1991.
- Ruf *et al.* discuss two sites in the tissue factor extracellular domain which mediate the recognition of the ligand factor VIIa. *Proc Natl Acad Sci* USA 88 (19): 8430-8434, 1991.
- Ruf *et al.* disclose antibody mapping of tissue factor implicates two different exon-encoded regions in function. *Biochem J* 278: 729-733, 1991.
- Ruf *et al.* disclose phospholipid-independent and dependent interactions which are required for TF Receptor and cofactor function. *Journal of Biol Chem,* 266 (4): 2158-2166, 1991.
- Ruf *et al.* disclose the mutational mapping of functional residues for tissue factor identification of factor VII recognition determinants in both structural modules of the predicted cytokine receptor homology domain. *Biochemistry,* 33 (6): 1565-1572, 1994.
- Schullek *et al.* disclose that key ligand interface residues in tissue factor contribute independently to factor VIIa binding. *J Biol Chem,* 269 (30): 19399-19403, 1994.

- Spicer *et al.* disclose the isolation of cDNA clones coding for human tissue factor primary structure of the protein and cDNA. *Proc Natl Acad Sci USA*, 84 (15): 5148-5152, 1987.

Claims 7-17, and 20-32 stand rejected under 35 U.S.C. 112, first paragraph, as purportedly lacking enablement. The Office action states that it is highly unpredictable whether locally administration of a nucleic acid expressing TF could achieve the goal of therapeutic symptomatic intervention for all the diseases recited. Applicants respectfully traverse, and provide a detailed explanation of why the local administration of a nucleic acid expressing TF could be therapeutically successful, based on what is known in the art.

Enablement of TF-fragments

Extensive literature referring to both the structure and the structure-function relationship of TF was available at the time the invention was made. Further, it was in the general knowledge of the skilled art that the full protein is not required for the complete function of TF. The parts of the protein which are required for its function have been well characterised. Thus, the skilled artisan could obtain suitable functional fragments without undue burden using what was already well known in the art.

Applicants refer the Examiner to the references listed above. In particular, Applicants refer to Paborsky (1991), which disclosed which domains are required for TF activity and to O'Brien (1993) which further showed which parts of the protein the activity is dependent on (*see* page 11, last two lines of the left column and the last paragraph of the

right column). Thus, it was known which regions must be encompassed by a functional fragment.

Further, mapping of the amino acids (*see* Ruf *et al.*, 1991; Ruf and Edginton, 1991; O'Brien, 1993; Ruf *et al.*, 1994; Gibb *et al.*, 1994; Schulleck *et al.*, 1994) and crystal structure studies (*see* Harlos *et al.*, 1994; Hasselbacher *et al.*, 1995; Muller *et al.*, 1996; Banner *et al.*, 1996) show which residues and regions are important for the binding and/or catalytic function of TF. Thus, it was known in the art at which positions mutations or deletions might be tolerable without losing the function of TF.

As a result, the skilled artisan can easily practice the invention without undue experimentation, because the detailed structure being required for a functional TF has been described in the art.

Enablement of Methods of treatment

Although the diseases recited in the present application may have distinct etiologies and mechanisms that may impair wound healing, the local administration of a nucleic acid expressing TF achieves a therapeutic symptomatic intervention for all recited diseases. These recited diseases all impair wound healing, after a history of disturbed but still functional angiogenesis and wound healing. Clinically apparent states often occur after a disturbance of the regulation by exhausted and otherwise impaired processes. This well known principle can be expected for all the diseases claimed. Thus, ulcer healing does occur in diabetic patients as well as in patients with inflammatory bowel disease. Blood

vessel formation does occur in patients with occlusive vascular diseases and thrombosis. In such cases administration of TF will induce or enhance this physiological process.

Furthermore, it is known from pro-thrombotic diseases as well as from experimental coagulation, that induction of coagulation is difficult to achieve. Instead of coagulation, angiogenesis was observed in an experiment designed for therapeutic coagulation in tumors (*see* Folkman, 1996; submitted with the Information Disclosure Statement of July 3, 2002) and after TF gene transfer in animals no pro-coagulatory action was observed. Thus, it must be expected that pro-coagulatory activity, if occurring at all, is of no practical relevance, although it might cause side effects. A number of studies have confirmed this principle and the teaching of the present invention. These include Watanabe *et al.* (1999), which disclose that TF directly induces angiogenesis independent of the coagulant pathway. Ollivier *et al.* (1998) disclose that TF has a role in endothelial recovery after vascular damage (*see* page 2702, right column, 2nd paragraph).

Furthermore, many examples in the literature show that different etiologies and mechanism that lead to impaired wound healing and/or impaired vessel formation are of no relevance, as long as the physiological process of blood vessel formation and/or wound healing can be induced by a method such as the use of a growth factor like VEGF or FGF. This is illustrated by the ample use of VEGF and other growth factors in therapeutic angiogenesis (*see* Brower, *Nat. Biotechn* Vol.17, 1999).

TF can trigger several events, only one of which is coagulation. In the recited diseases, the pro-angiogenic activity of TF promotes the pro-coagulant activity. In these conditions and for the claimed methods, the pro-coagulatory activity is not of concern to

the skilled artisan with regard to the outcome of the claimed methods (*see* claims 30 and 31 and 33 and 34) for two reasons. These are (1) overexpression of tissue factor is limited (timely and locally) and (2) coagulation is either beneficial (as in impaired wound healing) or has already occurred as part of the pathology at the site of the application, thus adding nothing extra disadvantageous (like death of the blood vessels) to the patient if TF really induced clot formation beside its blood vessel formation action.

Consequently, recent studies and publications support the teaching of the present application. The specification sufficiently enables a skilled person to practice the invention without undue experimentation. As discussed above, the pro-coagulatory activity of TF is not of concern for the claimed methods and, therefore, would not prevent a skilled person to apply the invention to the recited conditions.

Rejections under 35 U.S.C. § 102 and §103

Claims 7-10, 13-17, and 20-25 stand rejected under 35 U.S.C. § 102(e) as anticipated by *McDonald et al* (US 6,120,799). In the interest of expediting prosecution, the claims have been amended herein to recite the treatment of non-malignant tissue. In addition, the claims have been amended herein to recite the activating of blood vessel formation.

To anticipate a claim, a single source must contain all of the elements of the claim. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986). Applicants submit that this is not the case here. McDonald neither discloses nor teaches an activation of blood vessel formation. McDonald assumes that

delivering of nucleotide constructs encoding a human TF would result in the death of the vessel and the surrounding tissue. Hence, the amended claims are not anticipated by McDonald.

The Office Action further states that McDonald *et al.* recites that " the presence of tissue factor in a tissue with blood vessels would result in the formation of blood clots prevent the flow of nutrients and oxygen to the remainder of the vessel, resulting in the death of the vessel and the surrounding tissue" (*see* column 22, lines 1-7). Applicants submit that following evidence that the cited reference is incorrect. A large amount of evidence has accumulated showing that TF exerts multiple functions and pathways. In support, Applicants cite to the following references:

- Corseaux *et al.* discloses that basic fibroblast growth factor increases tissue factor expression in circulating monocytes and in vascular wall. Circulation 101 (16): 2000-2006, 2000.
- Connolly discloses that vascular permeability factor as a regulator of blood vessel function. *J Cell Biochem*, 47(3): 219-223, 1991.
- Mechtcheriakova *et al.*, disclose that induced TF expression in endothelial cells is mediated by EGR-1. *Blood,* 93(11): 3811-3823, 1999.
- Ollivier *et al.* disclose TF-dependent vascular endothelial growth factor production by human fibroblasts in response to activated Factor VII. *Blood*, 91(8): 2698-2703, 1998.

Watanabe *et al.* disclose angiogenesis induced by TF *in vitro* and *in vivo*. *Thrombosis Research* 96: 183-189, 1999.

- Poulsen *et al.* disclose signal transduction via the mitogen-activated protein kinase pathway induced by binding of coagulation Factor VIIa to Tissue Factor. *Journal of Biological Chemistry*, 273 (11): 6228-6232, 1998.

Watanabe *et al.* successfully demonstrated that tissue factor directly induces angiogenesis, independently of the coagulation pathway. *In vivo* angiogenesis was examined using a diffusion chamber assay in rats. The results demonstrated that after a week of implantation of the diffusion chambers containing tissue factor, angiogenesis was enhanced two to three times as compared with the control. *In vitro*, an addition of tissue factor enhanced angiogenesis in bovine aorta endothelial cells compared with the control, and the angiogenesis was inhibited by anti-TF antibody. Furthermore, tissue factor induced angiogenesis in bovine aorta endothelial cells was inhibited by the addition of coagulation factors II, VII, and IX. These results suggested that tissue factor directly induced angiogenesis in non-malignant cells independent of the coagulant system (Watanabe et al, 1999).

A further study supporting our statement was carried out by Paulsen *et al.* (1998) which suggests a specific mechanism by which binding of FVIIa to cell surface TF independent of coagulation can modulate cellular functions and therefore be involved in angiogenesis. Several studies have provided circumstantial evidence that FVIIa/TF may be involved in signal transduction and gene transcription. Poulsen *et al.* demonstrated in baby hamster kidney cells (BHK) that this is the case as it occurs in a FVIIa-and TF-dependent reaction via the p42/p44 mitogen-activated protein kinase (MAPK) pathway. The results

indicate that MAPK phosphorylation is a probable route to FVIIA-induced gene transcription. Therefore, FVIIa/TF-induced signal transduction might provide a common molecular mechanism linking the various cellular events, in which TF plays a crucial role, together. On the same note, Ollivier *et al.* carried out a study which was designed to link the binding of FVIIa to its receptor TF, with the subsequent triggering of angiogenesis through vascular endothelial growth factor (VEGF) production by human lung fibroblasts. VEGF is a well known major regulator of angiogenesis. The results demonstrated that tissue factor is essential for VIIa-induced VEGF production by human fibroblasts and that its role is mainly linked to the proteolytic activity of the TF-VIIa complex, as e.g. FVIIa-dependent VEGF production was inhibited by a pool of antibodies against tissue factor. Similarly, the treatment of endothelial cells with VEGF leads to the up regulation of tissue factor mRNA and protein expression on the cell surface (*see* Mechtcheriakova *et al.*). This study is supported by the findings of Connolly, who demonstrated that VEGF regulates the expression of tissue factor. Not only VEGF, also the basic fibroblast growth factor (FGF) which promotes vascular repair and angiogenesis was shown to induce tissue factor expression in circulating monocytes (*see* Corseaux et al).

Thus, a large amount of evidence is accumulating that demonstrates the involvement of tissue factor in the process of angiogenesis in various non-malignant cells and tissues.

Claims 7-17, and 20-27 stand rejected under 35 U.S.C. § 103(a) as unpatentable over *McDonald et al.* (US 6,120,799) as applied to claims 7-10, 13-17, and 20-25 above, and further in view of *Dubensky, Jr. et al.* (J Virology 1996 Jan; 70:508-1 9), for reasons

of record. Claims 7-10, 13-17, 20-25, and 28 stand rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over *McDonald et al.* (US 6,120,799) as applied to claims 7-10,13-17, and 20-25 above, and further in view of *Sanford et al.* (US 5,100,792), for reasons of record.

In response to the argument that the objective of the presently claimed invention is targeted induction of vessel formation in normal tissues, the Office Action states that the claims are not drawn to targeted vessel formation in normal tissue.

As indicated above, the claims have been amended herein to recite the targeted blood vessel formation in non-malignant tissue. Further, Applicants note that neither Dubensky nor Sanford disclose or even suggest TF. As McDonald does not disclose or suggest the use TF for activation blood vessel formation, the claimed invention is not rendered obvious over McDonald in the view of Dubensky or Sanford.

Applicants respectfully request that these rejections be withdrawn.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Attachment to Amendment and Reply

Marked-up Claims 7, 20, and 30-32

7. (Thrice Amended) A method of modulating blood vessel formation in a subject in need, comprising locally administering a functional tissue factor in a therapeutically effective amount to said subject in need, wherein said tissue factor or a fragment thereof is administered in the form of an expressible nucleic acid, and wherein the administration of the functional tissue factor modulates blood vessel formation in non-malignant tissue;

wherein the modulating is an activation of blood vessel formation.

20. (Amended) A method for modulating blood vessel formation in a subject in need, comprising inducing local expression of a tissue factor nucleic acid in said subject in need thereof;

wherein the modulating is an activation of blood vessel formation in non-malignant tissue.

30. (Amended) The method of claim 7 [29], wherein the said subject in need is afflicted with diabetis mellitus, vasculitis, arterial conclusive disease, chronic venous and infected ulcer, innervation impairment, decubital gangrene or weak sutures after a surgery.

Attachment to Amendment and Reply

Marked-up Claims 7, 20, and 30-32

31. (Amended) The method of claim ~~7~~ [29], wherein said subject in need is afflicted with arteriosclerosis, Crohn's disease, ulcerative colitis, diabetic retinopathy, or deep venous thrombosis of the legs ulcus cruris.

32. (Amended) The method of claim ~~7~~ [29], wherein the blood vessel formation is activated for the replacement of impaired blood vessels.